CLAIMS

We claim:

1. A method for the colocalization of two or more species of interest in a sample comprising:

a) exciting the species of interest with a single wavelength of light thereby causing the two or more species of interest to emit light of a distinctive emission characteristic,

b) separating the distinctive emission characteristics of the two or more species of interest,

c) detecting the emitted light from the two or more species of interest,

d) moving the sample a predetermined distance,

e) repeating steps a) through d) a predetermined number of times thereby creating a multitude of representations of the excitation point spread function (PSF),

f) determine the geometric center of the representations of the excitation PSF for at least two species of the two or more species of interest,

g) computing the distance between the geometric centers of the representations of the excitation PSF for the species of interest.

2. The method of claim 1, where the detection of the emitted light from the two or more species is accomplished either simultaneously or sequentially.
3. The method of claim 1, where the detection of the emitted light from the two or more species is accomplished simultaneously.
4. The method of claim 1, where the excitation of the species of interest is accomplished using a laser.
5. The method of claim 4, where the excitation of the species is a single photon process.
6. The method of claim 4, where the excitation of the species is a multiphoton process.
7. The method of claim 1, where the predetermined distance the sample is moved is a distance of from about 1 angstrom up to a distance of about 10 microns.
8. The method of claim 7, where the predetermined distance the sample is moved is about 25 nm.
9. The method of claim 1, where the emitted light is detected using one or more photodiodes or a photodiode array.

10. The method of claim 1, where the emitted light is detected using an ICCD or charge coupled device.

11. The method of claim 1, where separation is accomplished either by spectrum, polarization or lifetime.

12. The method of claim 1, where the separation is accomplished by spectrum.

13. The method of claim 1, where the determination of the geometric center of the representations of the excitation PSF for at least two of the species of interest is accomplished using an algorithm.

14. The method of claim 1, where the at least two species of interest comprise at least two fluorophores capable of being excited at the same wavelength.

15. The method of claim 1, where the at least two species of interest comprise at least two fluorophores which emit light at different wavelengths.

16. The method of claim 1, where the emitted light is directed through a confocal pinhole.

17. The method of claim 1, where the at least two species of interest comprise at least two fluorophores which emit light with different lifetimes.

18. The method of claim 1, where the emitted light is detected with digital technology or analog technology.

19. The method of claim 1, where the separation is based on a photophysical characteristic.

20. The method of claim 1, where the at least two species of interest comprise at least two fluorophores which emit light with different polarizations.

21. A apparatus for the colocalization of two or more species of interest in a sample comprising:

a scanner capable of supporting a sample and moving the sample,

a single wavelength light source capable of exciting the sample,

a device capable of separating the respective distinctive emission characteristics of the two or more species of interest,

a device capable of detecting the emitted light from the two or more species of interest.

22. The apparatus of claim 21, where the device capable of detecting the emitted light from the two or more species of interest detects emitted light simultaneously.

23. The apparatus of claim 21, where the light source is a laser.

24. The apparatus of claim 23, where the light source is a laser with wavelengths and power capable of exciting single photon processes.
25. The apparatus of claim 23, where the light source is a laser with wavelengths and power capable of exciting multiple photon processes.
26. The apparatus of claim 21, where the scanner is capable of moving a predetermined distance of from about 1 angstrom up to a distance of about 10 micron.
27. The apparatus of claim 21, where the scanner is capable of moving a predetermined distance of about 25 nm.
28. The apparatus of claim 21, where the device capable of detecting emitted light is a photodiode or a charge coupled device.
29. The apparatus of claim 23, where the laser is pulsed laser.
30. The method of claim 4, where the laser is a pulsed laser.
31. The method of claim 1, where the separation is based on differences in fluorescence lifetime.
32. The apparatus of claim 21, where the scanner is a closed-loop, 3 axis, piezo-scanner.
33. The apparatus of claim 21, where the scanner is an open-loop scanner.
34. The apparatus of claim 21, where the scanner performs a two dimensional scan.
35. The apparatus of claim 21, where the scanner performs a three dimensional scan.
36. The apparatus of claim 21, wherein there is included a confocal pinhole.
37. The method of claim 1, where the sample is moved three dimensionally.
38. The method of claim 1, where the sample is moved two dimensionally.
39. The method of claim 1, where the emitted light.
40. The method of claim 1, where the emitted light is detected using a point detector.
41. The apparatus of claim 21, where the device capable of detecting the emitted light is a point detector.